PCT

WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

B3

(51) International Patent Classification 6 :
C07H 21/04, A61K 38/08, 38/16, 39/395, C07K 14/705, 16/28, C12P 21/08, A01N 43/04

A1

(11) International Publication Number: WO 98/25946

(43) International Publication Date: 18 June 1998 (18.06.98)

(21) International Application Number: PCT/US97/20006

(22) International Filing Date: 5 November 1997 (05.11.97)

(30) Priority Data:
9604731-1 12 December 1996 (12.12.96) SE

(71) Applicant *(for all designated States except US)*: IMCLONE SYSTEMS INCORPORATED [US/US]; 180 Varick Street, New York, NY 10014 (US).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: DEJANA, Elisabetta [IT/IT]; Via Romolo Gessi, 48, I-20146 Milano (IT). TELO, Paola [IT/IT]; Via Carlo Goldoni, 8, I-25063 Gardone Val Trompia (IT).

(74) Agent: GALLAGHER, Thomas, C.; ImClone Systems Incorporated, 180 Varick Street, New York, NY 10014 (US).

(81) Designated States: AU, CA, CN, FI, HU, JP, KR, MX, NO, RU, SE, US, European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).

Published
With international search report.

(54) Title: MONOCLONAL ANTIBODIES SPECIFIC TO ENDOTHELIAL CELL CADHERINS AND USES THEREOF

(57) Abstract

A new VE-cadherin molecule is provided. Monoclonal antibodies that specifically bind to and neutralize an extracellular domain of a VE-cadherin molecule are provided. *In vitro* and *in vivo* methods of using these antibodies are also provided.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT

AL	Albania	ES	Spain	LS	Lesotho	SI	Slovenia
AM	Armenia	FI	Finland	LT	Lithuania	SK	Slovakia
AT	Austria	FR	France	LU	Luxembourg	SN	Senegal
AU	Australia	GA	Gabon	LV	Latvia	SZ	Swaziland
AZ	Azerbaijan	GB	United Kingdom	MC	Monaco	TD	Chad
BA	Bosnia and Herzegovina	GE	Georgia	MD	Republic of Moldova	TG	Togo
BB	Barbados	GH	Ghana	MG	Madagascar	TJ	Tajikistan
BE	Belgium	GN	Guinea	MK	The former Yugoslav Republic of Macedonia	TM	Turkmenistan
BF	Burkina Faso	GR	Greece			TR	Turkey
BG	Bulgaria	HU	Hungary	ML	Mali	TT	Trinidad and Tobago
BJ	Benin	IE	Ireland	MN	Mongolia	UA	Ukraine
BR	Brazil	IL	Israel	MR	Mauritania	UG	Uganda
BY	Belarus	IS	Iceland	MW	Malawi	US	United States of America
CA	Canada	IT	Italy	MX	Mexico	UZ	Uzbekistan
CF	Central African Republic	JP	Japan	NE	Niger	VN	Viet Nam
CG	Congo	KE	Kenya	NL	Netherlands	YU	Yugoslavia
CH	Switzerland	KG	Kyrgyzstan	NO	Norway	ZW	Zimbabwe
CI	Côte d'Ivoire	KP	Democratic People's Republic of Korea	NZ	New Zealand		
CM	Cameroon			PL	Poland		
CN	China	KR	Republic of Korea	PT	Portugal		
CU	Cuba	KZ	Kazakstan	RO	Romania		
CZ	Czech Republic	LC	Saint Lucia	RU	Russian Federation		
DE	Germany	LI	Liechtenstein	SD	Sudan		
DK	Denmark	LK	Sri Lanka	SE	Sweden		
EE	Estonia	LR	Liberia	SG	Singapore		

MONOCLONAL ANTIBODIES SPECIFIC TO ENDOTHELIAL CELL CADHERINS AND USES THEREOF

BACKGROUND OF THE INVENTION

Endothelial cells constitute an important interface lining the internal vascular surface and regulating the passage of plasma proteins and circulating cells from blood to tissues. (Caveda et al., J. Clin. Invest. 98(4): 886-893, August, 1996).

Endothelial permeability is regulated by intercellular junctions. These junctions are complex structures formed by transmembrane adhesive molecules, such as cadherins, linked to a network of cytoplasmic and cytoskeletal proteins. Adhesive molecules regulate leukocyte extravasation, endothelial cell growth, and permeability. (Dejana, E. et al , Review: Endothelial Cell-to-Cell Junctions, FASEB J., 9:910-918 (1995). Cadherins are adhesive glycoproteins that mediate homotypic cell-to-cell adhesion, are calcium-dependent, and protease-sensitive. All cell types that form solid tissues express some members of the cadherin family and each member displays a homophilic binding specificity. Members of the cadherin superfamily share a common basic structure. The common structures of cadherins include an N-terminal extracellular domain that determines binding specificity; a hydrophobic transmembrane domain; and a C-terminal cytoplasmic domain. The C-terminal cytoplasmic domain, which is highly conserved among the superfamily members, interacts with the cytoskeleton through catenins and other proteins. Some cadherins, however, lack a cytoplasmic domain. The most important biological role of cadherins is to support homotypic cell aggregation and segregation, which during embryogenesis promote the formation of defined tissues and organs. (Brevario, F., et al., Arterioscler. Thromb. Vasc. Biol. 15:1229-1239 (1995)).

Despite their similar biochemical properties, each cadherin is characterized by a different spatiotemporal pattern of expression and cell binding specificity. For

example, in humans, E-cadherin, or uvomorulin, is essentially found in epithelia and in subsets of neurons. N-cadherin is expressed in the nervous system and in skeletal and cardiac muscles, and P-cadherin exhibits a widespread distribution. (Takeichi, M., Annu. Rev. Biochem. 59:237-252 (1990)). Vascular endothelial cadherins (VE-cadherins) are endothelial-specific cadherins strictly localized at intercellular junctions of essentially all types of endothelium. (Brevario, F., et al. 1995) VE-cadherins are so far the only cadherins consistently organized at interendothelial adherence junctions. VE-cadherins are constitutive components of all types of endothelia, have adhesive properties, restrict endothelial permeability, and mediate homotypic cell adhesion. (Caveda et al.) The amino acid sequence of one such VE-cadherin, identified in this specification as VE-cadherin-1 (also known as cadherin-5 or VE-cadherin, and described previously in Lampugnani, M. et al., J.Cell Biol. 118:1511-1522 (1992)) shows moderate homology with other members of the family, but differences in the cytoplasmic and extracellular domains (Suzuki, S. et al., Cell Regul. 2:261-270 (1991) and Brevario, F., et al. 1995) suggest a specific role for this molecule in cell-to-cell adhesion and in its interaction with cytoskeletal proteins. (Dejana et al., Review, FASEB, vol. 9 (1995)).

The fact that VE-cadherins are constitutive endothelial-specific markers distinguishes these molecules from the majority of other cadherins as well as other endothelial markers. With the exception of M-cadherin, which is specifically found in skeletal muscle cells, most of the other cadherins described are quite widespread and are simultaneously expressed in different cell types during development. Other endothelial markers have different features from VE-cadherins. Some of them, such as QH-1, PECAM-1, von Willebrand factor, CD34, and P-selectin are not strictly endothelial-specific but may be found in blood cells or hematopoietic precursors. Other markers depend on the functional state of the cells. For example, the receptor protein tyrosine kinases flk-1, tie-1 and tie-2/tek are developmentally regulated and their appearance is a function of the maturation of the cell. Another endothelial marker, Meca 32, is not ubiquitous, but can be found only in the microvasculature of some organs.

Unlike most endothelial markers, VE-cadherins are not found in blood cells or in hematopoietic precursors. The observation that VE-cadherins are constitutively expressed by the endothelium of most organs and tissues suggests that their biologic properties are required for the early assembly and integrity of blood vessels. (Breier, G. et al., Blood, 87(2)L630-641 (1996)).

Blood vessels are formed by vasculogenesis, a process during which a primary capillary plexus is formed that is remodeled either by fusion or regression, and angiogenesis (also called neovascularization), a process in which vasculature is formed by new vessels sprouting from preexisting vessels and invading the developing organ. (Breier et al. 1996) Angiogenesis is an important process in the menstrual cycle in the endometrium, in pregnancy, and during neonatal growth. Angiogenesis is also important in wound healing and in the pathogenesis of a large variety of clinical diseases including tissue inflammation, arthritis, tumor growth, diabetic retinopathy, and macular degeneration by neovascularization of the retina. These clinical manifestations associated with angiogenesis are referred to as angiogenic diseases. (Folkman et al., Science, 235:442-447 (1987). Angiogenesis is generally absent in healthy adult or mature tissues, although it does occur in wound healing and in the corpous luteum growth cycle. See, for example, Moses et al., Science, 248:1408-1410 (1990).

Angiogenesis is required for tumor proliferation because tumors need an adequate blood perfusion to obtain nutrients. Inhibition of angiogenesis by limiting vessel growth or selectively destroying proliferating endothelium would be a useful therapy for restricting tumor growth. Various methods of inhibiting angiogenesis have been proposed: (1) inhibition of the release of "angiogenic molecules" such as basic-FGF (basic fibroblast growth factor); (2) neutralization of angiogenic molecules, such as basic-FGF by the use of anti-basic -FGF antibodies; and (3) inhibition of endothelial cell response to angiogenic stimuli. This latter strategy has received attention, and Folkman et al., Cancer Biology, 3:89-96 (1992), have described several endothelial cell response inhibitors, including collagenase inhibitor, angiostatic steroids, fungal-derived angiogenesis

inhibitors, platelet factor 4, thrombospondin, arthritis drugs such as D-penicillamine and gold thiomalate, vitamin D_3 analogs, alpha-interferon, and others that might be used to inhibit angiogenesis. For additional proposed inhibitors of angiogenesis, see Blood et al., Bioch. Biophys. Acta., 1032:89-8 (1990), Moses et al., Science 248:1408-1410 (1990), Ingber et al., Lab. Invest., 59:44-51 (1988), and U.S. Patent Nos. 5,092,885; 5,112,946; 5,192,744; and 5,202,352. Other new inhibitors of angiogenesis include angiostatin and endostatin (O'Reilly et al., Cell, 88(2) 277-285). None of the inhibitors of angiogenesis described in these references are targeted at inhibition of cadherins.

An object of this invention is to provide membrane markers of proliferating endothelial cells, i.e., VE-cadherins, which are useful in quantifying the degree of angiogenesis, and thus as diagnostic tools in evaluating the invasive state and other properties of a tumor. A further object of this invention is to provide antibodies against VE-cadherins, which participate in angiogenesis. Another object of this invention is to use such antibodies against VE-cadherins to inhibit angiogenesis to treat or prevent angiogenic diseases, such as tumor angiogenesis, rheumatoid arthritis, diabetic retinopathy and psoriasis. Such antibodies against VE-cadherins can also be useful as diagnostic tools to evaluate the invasive state and properties of a tumor.

SUMMARY OF THE INVENTION

The present invention provides a glycosylated or unglycosylated protein comprising an amino-acid sequence shown in SEQ ID NO:1 or a homologous sequence having at least 70% homology to the sequence shown in SEQ ID NO:1.

The present invention provides monoclonal antibodies which specifically bind to VE-cadherin molecules and modify their activity.

Further, the invention provides a method of modifying VE-cadherin activity in endothelial cells comprising contacting the cells with a monoclonal antibody of the invention.

The invention also provides a method of inhibiting angiogenesis in a mammal comprising administering an effective amount of any one of the antibodies of the invention to the mammal. In addition, the invention provides a method of inhibiting tumor growth in a mammal comprising administering an effective amount of any one of the antibodies of the invention to the mammal.

The invention also provides a pharmaceutical composition comprising any one of the antibodies of the invention and a pharmaceutically acceptable carrier.

DETAILED DESCRIPTION OF THE INVENTION

The present invention provides new transmembrane cadherin proteins located at cell-to-cell junctions in endothelial cells. In this specification, these cadherin proteins, found on vascular endothelial cells, are called VE-cadherins. One such VE-cadherin protein is VE-cadherin-1 (also known as cadherin-5, as well as VE-cadherin), whose amino acid sequence is presented in Lampugnani, M. et al., J.Cell Biol. 118:1511-1522 (1992). A second VE-cadherin provided by this invention is called in this specification protocadherin-4 (pcdh-4); alternatively, pcdh-4 is called VE-cadherin-2. The VE-cadherins of the invention promote cell-to-cell homotypic adhesion and its expression is upregulated in proliferating endothelial cells in comparison to resting cells. The VE-cadherin proteins of this invention are preferably of human origin, but may also be found in other animals such as mice, rats, pigs, monkeys, sheep and goats.

The present invention provides a pcdh-4 protein in glycosylated or unglycosylated form comprising an amino-acid sequence selected from the

sequence SEQ ID NO: 1 and homologous sequences having at least 70% homology to the sequence SEQ ID NO: 1. The percentage of homology may for instance be at least 75%, 80% or as high as 85%, or even higher such as 90% or 95%, especially if the homologous sequence originates from a transmembrane protein of the same or closely related species. However, it is anticipated that proteins which have at least 70 % homology to the amino-acid sequence SEQ ID NO: 1 will share both diagnostic and medical properties to such a high degree that they can be used for the various applications of the present invention. Among such proteins may be included both naturally occurring analogs and variants of the same protein from the same or from different species as well as synthetic or recombinant equivalents of these proteins.

The synthetically or recombinantly produced proteins of the invention function as competitors in cell-cell adhesion processes at cell-to-cell junctions.

The DNA of the invention can be any DNA that encodes the protein of the invention. Such DNA can be genomic or synthetic.

Another aspect of the invention is directed to a cDNA sequence coding for a protein of the present invention. A specific embodiment of this aspect of the invention is the cDNA sequence of SEQ ID NO: 2 coding for the protein having the amino-acid sequence of SEQ ID NO: 1. The cDNA molecules of the invention may be used in gene therapy. For example, they may be used as oncosuppressors by transfection in carcinoma cells lacking this molecule.

A further aspect of the invention is directed to a structural gene coding for a protein of the present invention or a peptide derived from the protein. The structural gene may be used in the production of a protein or peptide of the invention. The flanking regions, such as promoter or leader sequences, are preferably chosen with regard to the expression system to be used to promote good production. Further, the codons used in the structural gene

may be selected with regard to the codons most frequently used by the selected expression host, in order to optimize the expression yield. For instance, if yeast is selected as the expression host, the codons may be optimized for yeast. The specific example of a structural gene of the invention is the protein coding region of a cDNA of the invention, namely the structural gene having the nucleotide sequence SEQ ID NO: 3 coding for the protein having the amino-acid sequence SEQ ID NO: 1.

The present invention is also directed to a recombinant protein or peptide expressed by a structural gene or a fragment of a gene provided by the invention.

The invention is further directed to a modifier of the homophilic binding of VE-cadherins at cell-to-cell junctions. The term "modifier" is to be interpreted broadly and comprises both inhibitors and activators of the binding of the VE-cadherins. In one embodiment, the modifiers of the invention either prevent or promote binding of pcdh-4 molecules at cell-to-cell junctions. In other embodiments, the modifiers of the invention either prevent or promote binding of VE-cadherin-1 (cadherin-5) or pcdh-4 (VE-cadherin-2) molecules at cell-to-cell junctions.

A modifier of the invention may be any ligand to the protein of the invention, or any ligand, which binds to the protein and has the ability to prevent or promote the homophilic binding of VE-cadherin proteins. For example, the modifier of the invention may have a structure which is complementary to a VE-cadherin protein of the invention or a part of the protein. Such a modifier of the invention may bind to a VE-cadherin protein of the invention. In a preferred embodiment of this aspect of the invention the modifier is selected from the group consisting of antibodies specifically binding to the protein according to the invention and inhibiting or inducing or promoting the homophilic binding of said protein, and homophilic-binding-inhibiting or -inducing proteins, peptides, peptidomimetics and organic molecule-ligands derived from the amino-acid

sequence of the protein according to the invention. The invention also includes antisense oligonucleotides based on the cDNA sequence encoding the proteins of the invention, which may be used in cancer therapy as modifiers of angiogenesis.

The present invention provides antibodies that bind specifically to a VE-cadherin protein molecule of the invention or to a part of the VE-cadherin. The antibodies of the invention may be polyclonal, but preferably are monoclonal and preferably bind to the extracellular domain of a VE-cadherin molecule. The VE-cadherin molecule may be any cadherin molecule that is an endothelial-specific cadherin localized at intercellular junctions of essentially all types of endothelium, and that has adhesive properties, restrict endothelial permeability, and mediate homotypic cell adhesion. In one embodiment of the invention, the VE-cadherin molecule is called VE-cadherin-1, previously described as cadherin-5 or VE-cadherin, whose amino acid sequence is presented in Lampugnani, M. et al., J.Cell Biol. 118:1511-1522 (1992)). In another embodiment of the invention, the VE-cadherin is pcdh-4, which is alternatively named VE-cadherin-2.

The antibodies of the invention modify the activity of a VE-cadherin molecule. One way of modifying such activity is by interfering with or preventing cell-to-cell binding of the VE-cadherin's extracellular binding domain. Another way of modifying such activity is by inducing or promoting such cell-to-cell binding. In one embodiment of this invention, the antibodies of the invention will either prevent or promote homophilic binding of VE-cadherin molecules at cell-to-cell junctions. Accordingly, modification of VE-cadherin activity encompasses both inhibition of and activation of VE-cadherin activity.

UTILITY

A. Modifying VE-cadherin activity

In Vivo:

Modification of VE-cadherin activity in a sample of endothelial cells may be performed *in vivo*. Such modification occurs when a modifier of the invention, preferably an antibody, is contacted with a VE-cadherin upon administering the modifier to a mammal.

Additional modifiers of the invention include, but are not limited to, peptides, peptidometics, and small molecules.

Methods of administration to a mammal include, for example, oral, intravenous, intraperitoneal, subcutaneous, or intramuscular administration.

This *in vivo* method is useful for inhibiting angiogenesis in a mammal. The *in vivo* neutralization method is a useful therapeutic method, such as for preventing or inhibiting angiogenesis associated with pathological conditions such as tumor growth in a mammal. Accordingly, the modifiers, and more specifically, the antibodies, of the invention are anti-angiogenic immunotherapeutic agents.

The methods of inhibiting angiogenesis and of inhibiting pathological conditions such as tumor growth in a mammal comprises administering an effective amount of any one of the invention's antibodies to a mammal or directly to a tumor within the mammal. The mammal is preferably human. This method is effective for treating subjects with carcinomas or sarcomas, preferably highly vascular tumors such as Kaposi's sarcoma, CNS neoplasms (capillary hemangioblastomas, meningiomas and cerebral metastases), melanomas, gastrointestinal and renal sarcomas, rhabdomyosarcoma, glioblastoma, preferably glioblastoma multiforma, and leiomyosarcoma.

A cocktail of at least two monoclonal antibodies of the invention provides an especially efficient treatment for inhibiting angiogenesis and thus the growth of tumor cells. Any number of antibodies that is effective may be used, the upper

limit is determined by cost; preferably 10, more preferably 6, and most preferably not higher than 4.

The combined treatment of one or more of the antibodies of the invention with an anti-neoplastic or anti-chemotherapeutic drug such as doxorubicin, cisplatin or taxol provides an efficient treatment for inhibiting the growth of tumor cells. In one embodiment, the pharmaceutical composition comprises the antibody and carrier with an anti-chemotherapeutic drug attached thereto.

Preventing or inhibiting angiogenesis is also useful to treat non-neoplastic angiogenic pathologic conditions such as neovascular glaucoma, proliferative retinopathy including proliferative diabetic retinopathy, macular degeneration, hemangiomas, angiofibromas, and psoriasis.

In addition to prevention or inhibition of angiogenesis, other applications of the modifiers of the invention include the prevention or inhibition of leukocyte infiltration, tumor cell metastasis, or endothelial permeability. Further applications include using the modifiers as vaccines and for making endothelial junctions more permeable to antigens, thus indicating use of the modifiers for treatment or prevention of acute and chronic inflammatory diseases, organ transplantation, myocardial ischemia, atherosclerosis, cancer, diabetic retinopathy, psoriasis, rheumatoid arthritis, and intestinal infection.

A further application of the invention is that the antibodies of the invention may be labeled and used for detecting early endothelial cell damage *in vivo*. Additionally, the labeled antibodies can be used to detect and/or isolate cells that express the VE-cadherin molecules both *in vivo* and *in vitro*. Standard methods for labeling and using labeled antibodies are know in the art, such as standard blot and ELISA formats. These formats are normally based on incubating an antibody with a sample suspected of containing the protein and detecting the presence of a complex between the antibody and the protein. The antibody is labeled either before, during, or after the incubation step. The protein is

preferably immobilized prior to detection. Immobilization may be accomplished by directly binding the protein to a solid surface, such as a microtiter well, or by binding the protein to immobilized antibodies. (R.H. Kenneth, "Enzyme-Linked Antibody Assay with Cells Attached to Polyvinyl Chloride Plates" in Kenneth et al, Monoclonal Antibodies, Plenum Press, N.Y., page 376 (1981).)

In Vitro:

The invention provides a method of modifying VE-cadherin activity in a sample of endothelial cells comprising contacting the sample with an antibody of the invention before, simultaneously with, or after, adding VE-cadherin to the cell sample.

B. Using the Antibodies of the Invention to Isolate and Purify the VE-Cadherins and VE-Cadherin Expressing Cells

The antibodies of the present invention may be used to isolate and purify VE-cadherins, and cells expressing VE-cadherins, using conventional methods such as affinity chromatography (Dean, P.D.G. et al., Affinity Chromatography: A Practical Approach, IRL Press, Arlington, VA (1985)). Other methods well known in the art include magnetic separation with antibody-coated magnetic beads, "panning" with an antibody attached to a solid matrix, and flow cytometry.

The source of VE-cadherins is typically vascular endothelial cells, which express VE-cadherins. Suitable sources of vascular endothelial cells are blood vessels. The VE-cadherins may be used as starting material to produce other materials, such as DNA encoding the cadherins, or as antigen for making additional monoclonal and polyclonal antibodies that recognize and bind to the VE-cadherin or other related antigens on endothelial cells.

Modifiers of VE-cadherin-1, particularly monoclonal antibodies made against VE-cadherin-1, can bind to the extracellular domain of the protein. In some

cases the antibodies bind between amino acid residues 343 and 351. These antibodies block angiogenesis. The specific amino acid sequence to which these antibodies bind is TIDLRYMSP.

C. Monitoring Levels of VE-Cadherin *In Vitro* or *In Vivo*

The antibodies of the invention may be used to monitor levels of VE-cadherin *in vitro* or *in vivo* in biological samples using standard assays and methods known in the art. Some examples of biological samples include solid tissues, such as vascular tissue. Standard assays involve, for example, labeling the antibodies and conducting standard immunoassays, such as enzyme linked immunosorbent assays (ELISA) and radioimmunoassays, as is well known in the art. A preferred embodiment of the invention is a diagnostic kit comprising as a diagnostic reagent an antibody according to the invention or a modifier according to the invention. The actual diagnostic method, such as ELISA, to be used will determine any additional components in the kit.

The invention also provides transgenic animals or cells overexpressing or lacking a VE-cadherin protein. Transgenic animals carrying null mutation of pcdh-4 created by standard techniques, such as the knock-in and knock-out methods. Some examples of transgenic animals are those described by Hogan, B., et. al. , 1994. These transgenic animals may be used as *in vivo* models for screening replacing, activating molecules for VE-cadherins such as pcdh-4, and for providing the therapeutic potential of such cadherins in gene therapy in medicine. Transgenic animals may be engineered to overexpress by using promoters selected from NSE, Thy 1, PDGFB, VE cadherin, Willebrand factor, and transomodulin. Such transgenic animals can be used for screening *in vivo* for the therapeutic use of modifiers of VE-cadherin homophilic binding. Transgenic VE-cadherin cells may be used for *in vitro* testing purposes.

PREPARATION OF ANTIBODIES

The polyclonal and monoclonal antibodies of the invention that specifically bind to the VE-cadherins may be produced by methods known in the art. These methods include the immunological method described by Kohler and Milstein in Nature 256, 495-497 (1975) and Campbell in "Monoclonal Antibody Technology, The Production and Characterization of Rodent and Human Hybridomas" in Burdon et al., Eds., Laboratory Techniques in Biochemistry and Molecular Biology, Volume 13, Elsevier Science Publishers, Amsterdam (1985); as well as by the recombinant DNA method described by Huse et al in Science 246, 1275-1281 (1989).

Such antibody techniques include immunizing an animal, preferably a mouse, with an amount of a VE-cadherin molecule to cause an immune response. The spleen of an immunized animal, which demonstrates a proper antibody titre, is removed and a fused with an immortal cell line such as a myeloma cell line. The resultant hybridoma line is then screened for antibody producing cells; said cells are then clonally isolated.

The antibody may be prepared in any mammal, including mice, rats, rabbits, goats and humans. The antibody may be a member of one of the following immunoglobulin classes: IgG, IgM, IgA, IgD, or IgE, and the subclasses thereof, and preferably is an IgG antibody.

Functional Equivalents of Antibodies

The invention also includes functional equivalents of the antibodies described in this specification. Functional equivalents have binding characteristics comparable to those of the antibodies, and include, for example, chimerized, humanized and single chain antibodies as well as fragments thereof. Diabodies may also be functional equivalents of the antibodies of this invention. Methods of producing such functional equivalents are disclosed in PCT Application No. WO

93/21319, European Patent Application No. EPO 239,400; PCT Application Wo 89/09622; European Patent Application No. 338,745; and European Patent Application EPO 332,424.

Functional equivalents include polypeptides with amino acid sequences substantially the same as the amino acid sequence of the variable or hypervariable regions of the antibodies of the invention. "Substantially the same" amino acid sequence is defined herein as a sequence with at least 70% percent homology to an amino acid sequence of an antibody of the invention, as determined by the FASTA search method in accordance with Pearson and Lipman, Proc. Natl. Acad. Sci. USA 85, 2444-2448 (1988).

Chimerized antibodies preferably have constant regions derived substantially or exclusively from human antibody constant regions and variable regions derived substantially or exclusively from the sequence of the variable region from a mammal other than a human.

Humanized antibodies are commonly created by transplanting the antigen binding segments, known as complementarity determining regions (CDRs), from rodent antibodies into human antibodies. (Carter and Merchant, Current Opinions in Biotechnology (8):449-454, 1997.) Humanized antibodies preferably have constant regions and variable regions other than the hypervariable region derived substantially or exclusively from the corresponding human antibody regions and complementarity determining regions (CDRs) derived substantially or exclusively from a mammal other than a human. The extent to which an antibody is substantially or exclusively modified can be determined by standard methods for optimizing the humanization methodology.

Suitable mammals other than a human include any mammal from which monoclonal antibodies may be made, such as a rabbit, rat, mouse, horse, goat, or primate.

Single chain antibodies or Fv fragments (scFv) are polypeptides which consist of the variable (V) region of the heavy chain of the antibody linked to the variable (V) region of the light chain with or without an interconnecting linker. This comprises the entire antibody combining site, and is the minimal antibody binding site. These chains may be produced in bacteria.

Functional equivalents further include fragments of antibodies that have the same or binding characteristics comparable to those of the whole antibody. Such fragments may contain one or both Fab fragments or the $F(ab')_2$ fragment. Preferably the antibody fragments contain all six complementarity determining regions of the whole antibody, although fragments containing fewer than all of such regions, such as three, four or five CDRs, may also be functional.

Diabodies are examples of additional functional equivalents. A diabody is an antibody fragment which has two antigen binding sites and can be a bivalent or bispecific fragment. Bispecific diabodies are heterodimers of two 'crossover' scFv fragments in which the variable light and variable heavy domains of the two antibodies are present on different polypeptide chains. (Carter and Merchant, Current Opinions in Biotechnology (8):449-454, 1997.)

Further, the functional equivalents may be or may combine members of any one of the following immunoglobulin classes: IgG, IgM, IgA, IgD, or IgE, and the subclasses thereof.

Intracellularly expressed antibodies, referred to as "intrabodies" can be designed to bind and inactivate target molecules inside cells. The genes encoding can be expressed intracellularly. The specific and high-affinity binding properties of antibodies, combined with their ability to be stably expressed in precise intracellular locations inside mammalian cells, provides a molecules for gene therapy applications. (Marasco, W., Gene Ther (4) 1, p11-5, 1997).

Preparation of VE-Cadherin Immunogens

The VE-cadherins of the invention may be used as immunogens against which an antibody can be raised, particularly the antibodies of the invention. Alternatively, antibodies can be generated using as immunogens both synthetic peptides and VE-cadherin fragments. Such fragments and synthetic peptides are provided by the VE-cadherin amino acid sequences provided herein and by, for example, Lampugnani, M. et al., J.Cell Biol. 118:1511-1522 (1992)).

As a further alternative, DNA encoding a VE-cadherin, such as a cDNA or a fragment thereof, may be cloned and expressed and the resulting polypeptide recovered and used as an immunogen to raise an antibody of the invention. In order to prepare the VE-cadherins against which the antibodies are made, nucleic acid molecules that encode the VE-cadherins of the invention, or portions thereof, especially the extracellular portions thereof, may be inserted into known vectors for expression in host cells using standard recombinant DNA techniques. Standard recombinant DNA techniques are described in Sambrook et al., "Molecular Cloning," Second Edition, Cold Spring Harbor Laboratory Press (1987) and by Ausubel et al. (Eds) "Current Protocols in Molecular Biology," Green Publishing Associates/ Wiley-Interscience, New York (1990).

A suitable source of cells containing nucleic acid molecules that express the VE-cadherin includes vascular endothelial cells.

Total RNA or mRNA is prepared by standard procedures from endothelial tissue, or alternatively, from isolated endothelial cells. Standard methods may be used for Isolation of endothelial cells.

The total RNA or mRNA is used to direct cDNA synthesis. Standard methods for isolating RNA and synthesizing cDNA are provided in standard manuals of molecular biology such as, for example, in Sambrook et al., "Molecular Cloning," Second Edition, Cold Spring Harbor Laboratory Press (1987) and in Ausubel et

al., (Eds), "Current Protocols in Molecular Biology," Greene Associates/Wiley Interscience, New York (1990).

The cDNA of the VE-cadherin may be amplified by known methods. For example, the cDNA may be used as a template for amplification by polymerase chain reaction (PCR); see Saiki et al., Science, 239, 487 (1988) or Mullis et al., U.S. patent 4,683,195. The sequences of the oligonucleotide primers for the PCR amplification are derived from the sequences of mouse and human VE-cadherin respectively. The oligonucleotides are synthesized by methods known in the art. Suitable methods include those described by Caruthers in Science 230, 281-285 (1985).

In order to isolate the entire protein-coding regions for the VE-cadherins, the upstream PCR oligonucleotide primer is complementary to the sequence at the 5' end, preferably encompassing the ATG start codon and at least 5-10 nucleotides upstream of the start codon. The downstream PCR oligonucleotide primer is complementary to the sequence at the 3' end of the desired DNA sequence. The desired DNA sequence preferably encodes the entire extracellular portion of the VE-cadherin, and optionally encodes all or part of the transmembrane region, and/or all or part of the intracellular region, including the stop codon. A mixture of upstream and downstream oligonucleotides are used in the PCR amplification. The conditions are optimized for each particular primer pair according to standard procedures. The PCR product is analyzed by electrophoresis for cDNA having the correct size, corresponding to the sequence between the primers.

Alternatively, the coding region may be amplified in two or more overlapping fragments. The overlapping fragments are designed to include a restriction site permitting the assembly of the intact cDNA from the fragments.

The DNA encoding the VE-cadherins may also be replicated in a wide variety of cloning vectors in a wide variety of host cells. The host cell may be prokaryotic or eukaryotic.

The vector into which the DNA is spliced may comprise segments of chromosomal, non-chromosomal and synthetic DNA sequences. Some suitable prokaryotic cloning vectors include plasmids from E. coli, such as colE1, pCR1, pBR322, pMB9, pUC, pKSM, and RP4. Prokaryotic vectors also include derivatives of phage DNA such as M13 and other filamentous single-stranded DNA phages.

Expression and Isolation of VE-Cadherin Immunogens

DNA encoding the VE-cadherins of the invention are inserted into a suitable expression vector and expressed in a suitable prokaryotic or eucaryotic host. The DNA inserted into a host may encode the entire extracellular portion of the VE-cadherin, or a soluble fragment of the extracellular portion of the VE-cadherin. The extracellular portion of the VE-cadherin encoded by the DNA is optionally attached at either, or both, the 5' end or the 3' end to additional amino acid sequences. The additional amino acid sequence may be attached to the VE-cadherin extracellular region in nature, such as the leader sequence, the transmembrane region and/or the intracellular region of the VE-cadherin. The additional amino acid sequences may also be sequences not attached to the VE-cadherin in nature. Preferably, such additional amino acid sequences serve a particular purpose, such as to improve expression levels, secretion, solubility, or immunogenicity.

Vectors for expressing proteins in bacteria, especially *E. coli*, are known. Such vectors include the PATH vectors described by Dieckmann and Tzagoloff in J. Biol. Chem. 260, 1513-1520 (1985). These vectors contain DNA sequences that encode anthranilate synthetase (TrpE) followed by a polylinker at the carboxy terminus. Other expression vector systems are based on beta-galactosidase

(pEX); lambda P_L; maltose binding protein (pMAL); and glutathione S-transferase (pGST) -see Gene 67, 31 (1988) and Peptide Research 3, 167 (1990).

Vectors useful in yeast are available. A suitable example is the 2μ plasmid.

Suitable vectors for use in mammalian cells are also known. Such vectors include well-known derivatives of SV-40, adenovirus, retrovirus-derived DNA sequences and shuttle vectors derived from combination of functional mammalian vectors, such as those described above, and functional plasmids and phage DNA.

Further eukaryotic expression vectors are known in the art (e.g., P.J. Southern and P. Berg, J. Mol. Appl. Genet. 1, 327-341 (1982); S. Subramani et al, Mol. Cell. Biol. 1, 854-864 (1981); R.J. Kaufmann and P.A. Sharp, "Amplification And Expression Of Sequences Cotransfected with A Modular Dihydrofolate Reductase Complementary DNA Gene," J. Mol. Biol. 159, 601-621 (1982); R.J. Kaufmann and P.A. Sharp, Mol. Cell. Biol. 159, 601-664 (1982); S.I. Scahill et al, "Expression And Characterization Of The Product Of A Human Immune Interferon DNA Gene In Chinese Hamster Ovary Cells," Proc. Natl. Acad. Sci. USA 80, 4654-4659 (1983); G. Urlaub and L.A. Chasin, Proc. Natl. Acad. Sci. USA 77, 4216-4220, (1980).

The expression vectors useful in the present invention contain at least one expression control sequence that is operatively linked to the DNA sequence or fragment to be expressed. The control sequence is inserted in the vector in order to control and to regulate the expression of the cloned DNA sequence. Examples of useful expression control sequences are the lac system, the trp system, the tac system, the trc system, major operator and promoter regions of phage lambda, the control region of fd coat protein, the glycolytic promoters of yeast, e.g., the promoter for 3-phosphoglycerate kinase, the promoters of yeast acid phosphatase, e.g., Pho5, the promoters of the yeast alpha-mating factors, and promoters derived from polyoma, adenovirus, retrovirus, and simian virus,

e.g., the early and late promoters or SV40, and other sequences known to control the expression of genes of prokaryotic or eukaryotic cells and their viruses or combinations thereof.

Vectors containing the receptor-encoding DNA and control signals are inserted into a host cell for expression of the receptor. Some useful expression host cells include well-known prokaryotic and eukaryotic cells. Some suitable prokaryotic hosts include, for example, E. coli, such as E. coli SG-936, E. coli HB 101, E. coli W3110, E. coli X1776, E. coli X2282, E. coli DHI, and E. coli MRCI, Pseudomonas, Bacillus, such as Bacillus subtilis, and Streptomyces. Suitable eukaryotic cells include yeast and other fungi, insect, animal cells, such as COS cells and CHO cells, human cells and plant cells in tissue culture.

Following expression in a host cell maintained in a suitable medium, the VE-cadherins may be isolated from the medium, and purified by methods known in the art. If the VE-cadherins are not secreted into the culture medium, the host cells are lysed prior to isolation and purification.

The antibodies of the invention may also be prepared from VE-cadherins expressed by endothelial cells, or alternatively a cell into which the DNA encoding a VE-cadherin has been transfected, such as 3T3 cells.

EXAMPLES

The Examples which follow are set forth to aid in understanding the invention but are not intended to, and should not be construed to, limit its scope in any way. The Examples do not include detailed descriptions of conventional methods employed in the construction of vectors and plasmids, the insertion of genes encoding polypeptides into such vectors and plasmids or the introduction of plasmids into hosts. Such methods are well known to those of ordinary skill in the art and are described in numerous publications including Sambrook, J., Fritsch, E.F. and Maniatis, T. (1989) Molecular Cloning: A Laboratory Manual, 2nd edition, Cold Spring Harbor Laboratory Press.

Identification of the cDNA of pcdh-4

Library Screening, DNA sequencing.

A lgt10 library from P4-P8 postnatal mouse brain capillary was screened as previously described (Breviario et al. 1992) using a 130 bp cDNA probe obtained by means of RT-PCR. RT-PCR was carried out using, as primers, degenerated oligonucleotides (Sano et al. 1993) and a cDNA preparation from endothelioma H5V cells (Garlanda et al. 1991). Plaques showing a strong positive hybridization signal were screened three times to obtain a single clone. Phage inserts were rescued in pBluescript vector and sequenced by use of the dideoxynucleotide chain termination method.

Generation of recombinant fragments and production of polyclonal antibodies

Recombinant fragments and polyclonal antibodies were produced in the laboratory using Qiaexpressionist Kit, Qiagen. The cDNA corresponding to EC1 (aa 74)-EC3 (recombinant fragment Extra 1) and to EC1-EC4 (recombinant fragment Extra 2) of pcdh-4 were prepared by PCR and subcloned into the BamHI-HindIII site of the expression vector pQE30 in the

correct reading frame. The plasmid DNAs were then introduced into M15 (pREP4) cells by a single-step transformation method. The fusion proteins were induced by the addition of IPTG and were purified from the extract by Ni-NTA resin affinity chromatography, as described by the manufacturer (Qiaexpressionist Kit, Qiagen).
Polyclonal antibodies against pcdh-4 were produced in rabbits by injecting 0.5 mg of the fusion protein in Freund's complete adjuvant at three subcutaneous sites. Subsequent injections were in Freund's incomplete adjuvant with 0.5 mg of the fusion protein. The resultant antibodies were purified with a protein A column.

Constructs and Transfections

Constructs: Preparation and transfection procedure were performed according to Breviario et al. 1995. Briefly, the mouse pcdh-4 cDNA cloned in pBluescript vector was cut with EcoRI, and the insert was subcloned into the pECE eucaryotic expression vector to give the pECE-pcdh-4 construct. The construct was checked for correct orientation by sequence analysis.
CHO cells were plated at 3-4x10^6 cells per 100 mm petri dish in DMEM with 10% FCS. After 24 hrs cells were transfected by calcium phosphate precipitation with 20 μg pECE-pcdh-4 and 2 μg pSV2neo plasmid. Medium was replaced by fresh medium 24 hours later and maintained for further 48 hrs. Then cells were detached and plated at 1x10^6 per 100 mm petri dish and cultured in selective medium with 600 μg/ml G418 (Geneticin, GIBCO). Resistant colonies were isolated and tested for pcdh-4 antigen expression by immunofluorescence staining and immunoprecipitation analysis. Positive cells were cloned by limiting dilution and expanded for further studies.

Localization of pcdh-4 at intercellular junctions

Immunofluorescence microscopy

Cells were seeded on glass coverslips and grown to confluence in D-MEM medium containing 10% fetal calf serum before immunofluorescence staining. Cells were fixed with MeOH for 4 min. and processed for indirect immunofluorescence microscopy as previously described in detail by Lampugnani et al. (1992). Briefly, incubation with the primary antibody (Extra1 or Extra 2 and others) was followed by rhodamine-conjugated secondary antibody (Dakopatts) with several washes with 0.1% BSA in PBS between the various steps. Coverslips were then mounted in Mowiol 4-88 (Calbiochem). A Zeiss Axiophot microscope was used for observation and image recording on Kodak TMax P3200 films.

Results

Pcdh-4 distributes selectively at cell-cell contacts in cultured mouse endothelial cells and in transfectant cells.

Localization of pcdh-4 in endothelial cells

Immunohistochemistry

Immunohistochemistry was performed according to Lampugnani et al. 1992. Tissue fragments were embedded in OCT compound (Ames Division), snap frozen in liquid nitrogen and stored at -80°C until sectioning. Cryostat sections were fixed in acetone for 10 min. at RT and were immunostained with the polyclonal antibodies Extra 1 or Extra 2 using avidin-biotin peroxidase complex technique. Sections were preincubated with horse serum to prevent non-specific binding, and then incubated with an optimal dilution of the primary antibody (1/50) for 30 min. The slides were sequentially incubated with biotin-conjugated horse anti-rabbit Ig antibodies followed by avidin-biotin peroxidase complex. Each incubation step lasted 30 min. with 5 min. TBS washes between each step. The sections were finally incubated with 0.03% H_2O_2 and 0.06% 3,3′ DAB for 3-5 min. Slides were then washed for 5 min. in running water, counterstained with hematoxilin for 5 min., and mounted in Canada balsam.

Results

Staining of different tissues indicate that pcdh-4 antibodies stain endothelial cells of the microvasculature, with higher staining intensity in proliferating vessels of tumors. Extra 1 also cross reacts with human tissues.

The polyclonal Ab Extra 1 can be used to detect pcdh-4 in ELISA assay

ELISA assay

Microtiter wells of confluent cells were washed three times with DMEM+2.5% horse serum +0.01% sodium azide and incubated for 1h at 37°C with 100 μl/well of rabbit anti-pcdh-4 serum, diluted 1/100 in PBS+2.5% horse serum. After incubation, cells were washed three times with PBS+2.5% horse serum (washing buffer). Then, cells were fixed with glutaraldehyde 0.025% in washing buffer for 5 min., washed two times and incubated for 1h with washing buffer. Fixation was required to prevent cells detaching from the culture wells during the following washes. After rinsing in washing buffer, cells were incubated with peroxidase conjugated anti-rabbit IgG (diluted 1/500 SIGMA) for 1h at RT. After incubation and three washes, 100 μl chromogen substrate was added. Absorbance values were read at 490 nm. In each experiment, the binding of peroxidase conjugated anti-rabbit IgG to the cells in the presence of non-immune serum was evaluated. This value, considered as background was subtracted from each measurement.

Results

In ELISA the Ab Extra 1 was able to detect pcdh-4 protein in endothelial cells (such as H5V from heart microvasculature; bEnd from brain microvasculature) and pcdh-4 transfectant cells, while it gave negative values using cells which do not express pcdh-4 such as CHO parental and L929 fibroblast.

The results are presented in Table 1.

Table 1. Detection of pcdh-4 on endothelial cells and transfectants in ELISA

Cell types	OD x 1000
H5V	305.3 ± 12*
bEnd	300.2 ± 18*
CHO-pcdh-4	380.5 ± 10*
CHO-parental	118.6 ± 6
L929	148.4 ± 5

Non-immune serum was used as dilution 1/100. Absorbance values are means ± SD of five replicates of a typical experiment out of four performed. *P<0.01 in comparison to CHO-parental by analysis of variance and Duncan's test.

<u>**The ab Extra 1 can be used to detect proliferating endothelial cells**</u>

ELISA assay was used to detect pcdh-4 in subconfluent proliferating endothelial cells 1.2×10^4 cells/cm^2 in comparison to non-proliferating confluent cells 1.2×10^5 cells/cm^2.

Results

Pcdh-4 expression is higher in endothelial cells in growth than in cells at confluence.

Results are presented in Table 2.

Table 2. Modulation of the expression of pcdh-4 at different stages of growth

H5V growth stage	OD x 1000
Subconfluents (1.2×10^4 cells/cm^2)	389.3 ± 2*
Confluents (1.2×10^5 cells/cm^2)	243.2 ± 4

Values are means ± SD of five replicates of a typical experiment out of four performed. *P<0.01 by Student's test.

Protocadherin 4 mediates homotypic adhesion between cells

Cell Aggregation

The procedure to measure cell aggregation is extensively described in Breviario et al. (1995). Briefly, confluent control cells, CHO transfected with the empty pECE and pSV2neo plasmids, and confluent pcdh-4-transfectants were washed several times with Ca++ and Mg++ -PBS. Then, 0.01% trypsin in Hank's balanced salt solution (HBSS) with 25mM HEPES, 10 mM Ca++ and 5 mM Mg++ was added and maintained on the cells for the shortest time interval before the appearance of intercellular retraction. Cells were completely detached by vigorous shaking of the flasks. Trypsin was neutralized by adding DMEM with 10% FCS and 0.1% soybean trypsin inhibitor. The cell suspensions were centrifuged and resuspended in HBSS without Ca++ and Mg++ and then centrifuged and resuspended in 1% BSA in HBSS Ca++ and Mg++ free at a concentration of 4×10^5 ml. Cell suspensions (0.5 ml per well) were seeded in a 24-well plate previously coated with 1% BSA to prevent cell adhesion, and treatments: 5 mM $CaCl_2$, 5mM EGTA, 50 μg/ml rabbit pcdh-4 purified antiserum (Extra 1), rabbit non-immune serum, were added to start aggregation. Controls without calcium addition were always run in parallel. Incubation was for 90 min. at 37°C on a rotating platform (80 rpm). The reaction was stopped with 5% glutaraldehyde. The initial number of particles (Nt0) and the number of particles at 90 min. (Nt90)

were counted using a ZM Coulter Counter. Aggregation was quantified by use of the formula (Nt0-Nt90)/Nt0 x 100.

For those experiments where anactin cytoskeleton disrupting agent was used, cytochalasin D was added at 1 μg/ml after the first centrifugation, and the cells were incubated at 37°C for 30 min. and processed as described.

Results

Only pcdh-4 transfectants showed significant calcium-dependent aggregation.

The aggregation capacity was lost when EGTA was added to the CHO-pcdh-4 suspension. The rabbit purified antiserum, generated using the recombinant fragment Extra 1, was able to neutralize the aggregation capacity of CHO-pcdh-4 cells. Cytochalasin D did not affect aggregation indicating that the formation of aggregates does not require an intact actin cytoskeleton.

The results are presented in Table 3.

Table 3. Effect of pcdh-4 transfection on cell aggregation capacity.

Transfectant cells	% Aggregation
CHO-parental	10 ± 2.0
CHO-pcdh-4	12 ± 4.0
CHO-pcdh-4+Ca 5mM	50 ± 8.2*
CHO-pcdh-4-Ca 5mM+EGTA 5 mM	15 ± 5.4
CHO-pcdh-4+Ca 5mM+Ab (Extra1)	22 ± 7.0
CHO-pcdh-4-Ca 5mM+non-immune serum	42 ± 6.5*
CHO-pcdh-4+Ca 5mM+Cyt D	55 ± 4.1*

Values are means ± SD from triplicates of a typical experiment out of three performed. *P<0.01 by analysis of variance and Duncan´s test in comparison to CHO parental.

Pcdh-4 binding was homophilic

Aggregation assays were performed after mixing parental CHO cells and pcdh-4 transfectants. To distinguish the two cell types, parental cells were labeled with 5 μg/ml of the fluorescent dye calcein in HBSS for 10 min. at 37°C, immediately after the first centrifugation and processed as described above. Aggregates were examined by fluorescence microscopy. The results showed that aggregation is essentially homophilic: only pcdh-4 transfectants were present in the aggregates whereas control cells remained mostly single.

Pcdh-4 promote homotypic cell adhesion

Cell Adhesion

Cells in monolayers were obtained by culturing control cells and CHO-pcdh-4 transfectants ($5x10^3$/well at the seeding) in 96-well plates for 4 - 5 days to confluence. Cells to be used in suspension were labeled 1h with ^{51}Cr ($1\mu Ci/10^6$ cells). Detachment was as described above for the cell aggregation assay. Labeled cell suspensions ($4x10^4$ cells in 100 μl DMEM with 10% FCS for each well) were added on the top of adherent cells (from which culture medium had been removed with no rinsing). Incubation was for 30 min. at 37°C. Non-adherent cells were removed by three washes with Ca++ and Mg++-PBS containing 10% FCS. The well content was then solubilized with 1 M NaOH/0.1% SDS (50 μl) and counted for radioactivity.

Results

CHO-pcdh-4 significantly adhered to homologous pcdh-4 transfectant monolayer, whereas parental cells adhered poorly to the transfectants. The results are presented in Table 4.

Table 4. Effect on pcdh-4 transfection on cell adhesion

Cell layer	Cell suspension	Number of adhered cells
CHO-parental	CHO-pcdh-4	4770 ± 950*
CHO-pcdh-4	CHO-pcdh-4	14800 ± 1800

Values are means ± SD of five replicates of a typical experiment out of two performed. *P<0.01 by Student's test.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT:

(A) NAME: Tamas Bartfai Konsulting AB

(B) STREET: Brinken 3

(C) CITY: Stocksund

(E) COUNTRY: Sweden

(F) POSTAL CODE (ZIP): 182 74

(ii) TITLE OF INVENTION: A component of intercellular junctions in the endothelium.

(iii) NUMBER OF SEQUENCES: 3

(iv) COMPUTER READABLE FORM:

(A) MEDIUM TYPE: Floppy disk

(B) COMPUTER: IBM PC compatible

(C) OPERATING SYSTEM: PC-DOS/MS-DOS

(D) SOFTWARE: PatentIn Release #1.0, Version #1.30 (EPO)

(2) INFORMATION FOR SEQ ID NO: 1:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 1180 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS:

(D) TOPOLOGY: both

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

```
Met Met Leu Leu Leu Pro Phe Leu Leu Gly Leu Leu Gly Pro Gly Ser
1               5                   10                  15
```

Tyr Leu Phe Ile Ser Gly Asp Cys Gln Glu Val Ala Thr Val Met Val
20 25 30

Lys Phe Gln Val Thr Glu Glu Val Pro Ser Gly Thr Val Ile Gly Lys
35 40 45

Leu Ser Gln Glu Leu Arg Val Glu Glu Arg Arg Gly Lys Ala Gly Asp
50 55 60

Ala Phe Gln Ile Leu Gln Leu Pro Gln Ala Leu Pro Val Gln Met Asn
65 70 75 80

Ser Glu Asp Gly Leu Leu Ser Thr Ser Ser Arg Leu Asp Arg Glu Lys
85 90 95

Leu Cys Arg Gln Glu Asp Pro Cys Leu Val Ser Phe Asp Val Leu Ala
100 105 110

Thr Gly Ala Ser Ala Leu Ile His Val Glu Ile Gln Val Leu Asp Ile
115 120 125

Asn Asp His Gln Pro Gln Phe Pro Lys Asp Glu Gln Glu Leu Glu Ile
130 135 140

Ser Glu Ser Ala Ser Leu His Thr Arg Ile Pro Leu Asp Arg Ala Leu
145 150 155 160

Asp Gln Asp Thr Gly Pro Asn Ser Leu Tyr Ser Tyr Ser Leu Ser Pro
165 170 175

Ser Glu His Phe Ala Leu Asp Val Ile Val Gly Pro Asp Glu Thr Lys
180 185 190

His Ala Glu Leu Val Val Val Lys Glu Leu Asp Arg Glu Leu His Ser
195 200 205

Tyr Phe Asp Leu Val Leu Thr Ala Tyr Asp Asn Gly Asn Pro Pro Lys
210 215 220

Ser Gly Ile Ser Val Val Lys Val Asn Val Leu Asp Ser Asn Asp Asn
225 230 235 240

Ser Pro Val Phe Ala Glu Ser Ser Leu Ala Leu Glu Ile Pro Glu Asp
245 250 255

Thr Val Pro Gly Thr Leu Leu Ile Asn Leu Thr Ala Thr Asp Pro Asp
260 265 270

Gln Gly Pro Asn Gly Glu Val Glu Phe Phe Phe Gly Lys His Val Ser
275 280 285

Pro Glu Val Met Asn Thr Phe Gly Ile Asp Ala Lys Thr Gly Gln Ile
290 295 300

Ile Leu Arg Gln Ala Leu Asp Tyr Glu Lys Asn Pro Ala Tyr Glu Val
305 310 315 320

Asp Val Gln Ala Arg Asp Leu Gly Pro Asn Ser Ile Pro Gly His Cys
325 330 335

Lys Val Leu Ile Lys Val Leu Asp Val Asn Asp Asn Ala Pro Ser Ile
340 345 350

Leu Ile Thr Trp Ala Ser Gln Thr Ser Leu Val Ser Glu Asp Leu Pro
355 360 365

Arg Asp Ser Phe Ile Ala Leu Val Ser Ala Asn Asp Leu Asp Ser Gly
370 375 380

Asn Asn Gly Leu Val His Cys Trp Leu Asn Gln Glu Leu Gly His Phe
385 390 395 400

Arg Leu Lys Arg Thr Asn Gly Asn Thr Tyr Met Leu Leu Thr Asn Ala
405 410 415

Thr Leu Asp Arg Glu Gln Trp Pro Ile Tyr Thr Leu Thr Val Phe Ala
420 425 430

Gln Asp Gln Gly Pro Gln Pro Leu Ser Ala Glu Lys Glu Leu Gln Ile

435 440 445

Gln Val Ser Asp Val Asn Asp Asn Ala Pro Val Phe Glu Lys Ser Arg
450 455 460

Tyr Glu Val Ser Thr Trp Glu Asn Asn Pro Pro Ser Leu His Leu Ile
465 470 475 480

Thr Leu Lys Ala His Asp Ala Asp Leu Gly Ser Asn Gly Lys Val Ser
485 490 495

Tyr Arg Ile Lys Asp Ser Pro Val Ser His Leu Val Ile Ile Asp Phe
500 505 510

Glu Thr Gly Glu Val Thr Ala Gln Arg Ser Leu Asp Tyr Glu Gln Met
515 520 525

Ala Gly Phe Glu Phe Gln Val Ile Ala Glu Asp Arg Gly Gln Pro Gln
530 535 540

Leu Ala Ser Ser Ile Ser Val Trp Val Ser Leu Leu Asp Ala Asn Asp
545 550 555 560

Asn Ala Pro Glu Val Ile Gln Pro Val Leu Ser Glu Gly Lys Ala Thr
565 570 575

Leu Ser Val Leu Val Asn Ala Ser Thr Gly His Leu Leu Leu Pro Ile
580 585 590

Glu Asn Pro Ser Gly Met Asp Pro Ala Gly Thr Gly Ile Pro Pro Lys
595 600 605

Ala Thr His Ser Pro Trp Ser Phe Leu Leu Leu Thr Ile Val Ala Arg
610 615 620

Asp Ala Asp Ser Gly Ala Asn Gly Glu Leu Phe Tyr Ser Ile Gln Ser
625 630 635 640

Gly Asn Asp Ala His Leu Phe Phe Leu Ser Pro Ser Leu Gly Gln Leu
645 650 655

Phe Ile Asn Val Thr Asn Ala Ser Ser Leu Ile Gly Ser Gln Trp Asp
660 665 670

Leu Gly Ile Val Val Glu Asp Gln Gly Ser Pro Ser Leu Gln Thr Gln
675 680 685

Val Ser Leu Lys Val Val Phe Val Thr Ser Val Asp His Leu Arg Asp
690 695 700

Ser Ala His Glu Pro Gly Val Leu Ser Thr Pro Ala Leu Ala Leu Ile
705 710 715 720

Cys Leu Ala Val Leu Leu Ala Ile Phe Gly Leu Leu Leu Ala Leu Phe
725 730 735

Val Ser Ile Cys Arg Thr Glu Arg Lys Asp Asn Arg Ala Tyr Asn Cys
740 745 750

Arg Glu Ala Glu Ser Ser Tyr Arg His Gln Pro Lys Arg Pro Gln Lys
755 760 765

His Ile Gln Lys Ala Asp Ile His Leu Val Pro Val Leu Arg Ala His
770 775 780

Glu Asn Glu Thr Asp Glu Val Arg Pro Ser His Lys Asp Thr Ser Lys
785 790 795 800

Glu Thr Leu Met Glu Ala Gly Trp Asp Ser Cys Leu Glu Ala Pro Phe
805 810 815

His Leu Thr Pro Thr Leu Tyr Arg Thr Leu Arg Asn Gln Gly Asn Gln
820 825 830

Gly Glu Leu Ala Glu Ser Gln Glu Val Leu Gln Asp Thr Phe Asn Phe
835 840 845

Leu Phe Asn His Pro Arg Gln Arg Asn Ala Ser Arg Glu Asn Leu Asn
850 855 860

Leu Pro Glu Ser Pro Pro Ala Val Arg Gln Pro Leu Leu Arg Pro Leu
865 870 875 880

Lys Val Pro Gly Ser Pro Ile Ala Arg Ala Thr Gly Asp Gln Asp Lys
885 890 895

Glu Glu Ala Pro Gln Ser Pro Pro Ala Ser Ser Ala Thr Leu Arg Arg
900 905 910

Gln Arg Asn Phe Asn Gly Lys Val Ser Pro Arg Gly Glu Ser Gly Pro
915 920 925

His Gln Ile Leu Arg Ser Leu Val Arg Leu Ser Val Ala Ala Phe Ala
930 935 940

Glu Arg Asn Pro Val Glu Glu Pro Ala Gly Asp Ser Pro Pro Val Gln
945 950 955 960

Gln Ile Ser Gln Leu Leu Ser Leu Leu His Gln Gly Gln Phe Gln Pro
965 970 975

Lys Pro Asn His Arg Gly Asn Lys Tyr Leu Ala Lys Pro Gly Gly Ser
980 985 990

Ser Arg Gly Thr Ile Pro Asp Thr Glu Gly Leu Val Gly Leu Lys Pro
995 1000 1005

Ser Gly Gln Ala Glu Pro Asp Leu Glu Glu Gly Pro Pro Ser Pro Glu
1010 1015 1020

Glu Asp Leu Ser Val Lys Arg Leu Leu Glu Glu Glu Leu Ser Ser Leu
1025 1030 1035 1040

Leu Asp Pro Asn Thr Gly Leu Ala Leu Asp Lys Leu Ser Pro Pro Asp
1045 1050 1055

Pro Ala Trp Met Ala Arg Leu Ser Leu Pro Leu Thr Thr Asn Tyr Arg
1060 1065 1070

Asp Asn Leu Ser Ser Pro Asp Ala Thr Thr Ser Glu Glu Pro Arg Thr

1075 1080 1085

Phe Gln Thr Phe Gly Lys Thr Val Gly Pro Gly Pro Glu Leu Ser Pro
1090 1095 1100

Thr Gly Thr Arg Leu Ala Ser Thr Phe Val Ser Glu Met Ser Ser Leu
1105 1110 1115 1120

Leu Glu Met Leu Leu Gly Gln His Thr Val Pro Val Glu Ala Ala Ser
1125 1130 1135

Ala Ala Leu Arg Arg Leu Ser Val Cys Gly Arg Thr Leu Ser Leu Asp
1140 1145 1150

Leu Ala Thr Ser Gly Ala Ser Ala Ser Glu Ala Gln Gly Arg Lys Lys
1155 1160 1165

Ala Ala Glu Ser Arg Leu Gly Cys Gly Arg Asn Leu
1170 1175 1180

(2) INFORMATION FOR SEQ ID NO: 2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 3868 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA to mRNA

(iii) HYPOTHETICAL: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

GCGGCCGCTG TCAAGTCTCT CTTAGACCGG TACTTGCCCA TCACTGCTAA GTGGACCAGC 60

TGGTGCTGTG GCGGAGCAGG AATCTCTTCC AGCAATTTAT CTGTTCTGGG ACCTCTCACT 120

TGTCACGGAG ATGGCCTTGG TTGGGAATCC TGCTCCTTAC AGCATCTTCT AATTATGGGA 180

CAGAGTTGTA ACAGCAGGTC TGAAGTGGGA GATCCAGGCT GACCAAGCCA TTCAGGAACT 240

ACTGGGGACA AGCTCTGCCT TTGAAAAACT CCAGTCCAGC CTACCTGCCG GTAAGCATGA 300

TGCTACTTCT GCCATTCCTG CTAGGGCTCT TAGGGCCAGG AAGCTACTTG TTCATTTCAG 360

GGGATTGTCA GGAGGTGGCC ACTGTCATGG TGAAATTCCA AGTGACAGAG GAAGTGCCGT 420

CTGGCACGGT GATAGGGAAA CTGTCCCAAG AACTAAGAGT GGAGGAGAGG CGTGGGAAGG 480

CAGGAGATGC CTTCCAGATT CTGCAGCTGC CTCAGGCACT GCCGGTTCAG ATGAACTCTG 540

AGGACGGCCT GCTCAGCACT TCCAGCCGGC TGGATCGGGA GAAGCTATGT CGGCAGGAAG 600

ATCCCTGTCT GGTGTCATTT GACGTGCTTG CCACAGGGGC GTCTGCTCTA ATTCATGTGG 660

AGATTCAGGT GCTAGACATC AATGACCACC AGCCACAGTT TCCCAAAGAC GAGCAGGAAC 720

TGGAAATCTC AGAGAGTGCC TCTCTGCACA CACGAATCCC CTTGGACAGA GCTCTTGACC 780

AAGACACGGG TCCTAACAGC TTATATTCCT ACTCCCTGTC TCCCAGTGAA CACTTTGCCC 840

TGGATGTTAT TGTGGGCCCT GATGAGACCA AACATGCAGA GCTTGTGGTG GTGAAGGAGT 900

TGGACAGGGA ACTCCACTCA TATTTTGATC TGGTGCTGAC CGCCTATGAC AATGGGAATC 960

CCCCTAAGTC AGGAATCAGC GTGGTCAAGG TCAATGTCCT GGACTCCAAT GACAATAGTC 1020

CAGTGTTTGC TGAGAGTTCA CTAGCACTAG AAATCCCAGA AGACACTGTT CCTGGTACTC 1080

TTCTCATAAA CCTGACTGCT ACAGATCCCG ACCAAGGACC CAATGGGGAG GTAGAGTTCT 1140

TCTTTGGCAA GCATGTGTCC CCAGAGGTGA TGAACACCTT TGGCATAGAT GCCAAGACAG 1200

GCCAGATCAT TCTGCGCCAA GCCCTAGATT ACGAGAAGAA CCCTGCCTAT GAGGTGGATG 1260

TCCAGGCAAG GGATTTGGGT CCCAATTCCA TCCCAGGCCA TTGCAAAGTT CTTATCAAAG 1320

TTCTGGATGT CAATGACAAT GCCCCAAGCA TCCTCATCAC GTGGGCCTCC CAGACGTCGC 1380

TGGTGTCAGA AGATCTTCCC AGGGATAGCT TCATTGCCCT TGTCAGTGCG AATGACTTGG 1440

ACTCAGGAAA CAACGGTCTC GTCCACTGTT GGCTGAATCA AGAGCTGGGC CACTTCAGAC 1500

TGAAAAGGAC TAACGGCAAC ACGTACATGC TGCTCACCAA TGCCACACTG GACAGAGAGC 1560

AGTGGCCCAT ATATACTCTC ACTGTGTTTG CCCAAGACCA AGGACCCCAG CCCTTATCAG 1620

CTGAGAAGGA GCTCCAAATT CAGGTTAGTG ATGTCAATGA CAATGCCCCT GTGTTTGAGA 1680

AGAGCCGGTA CGAGGTCTCC ACTTGGGAAA ATAACCCACC CTCTCTTCAC CTCATCACGC 1740

TCAAAGCGCA TGATGCTGAC TTGGGCAGTA ATGGAAAAGT GTCATACCGT ATCAAGGACT 1800

CCCCCGTTTC TCACTTAGTC ATTATTGACT TTGAAACAGG AGAAGTCACT GCTCAGAGGT 1860

CACTGGACTA TGAACAGATG GCAGGCTTTG AGTTCCAGGT GATAGCAGAG GACAGAGGGC 1920

AACCCCAGCT CGCATCCAGC ATCTCGGTGT GGGTTAGCCT CTTGGATGCC AATGATAATG 1980

CCCCAGAAGT GATTCAGCCT GTGCTCAGTG AAGGCAAAGC CACCCTTTCG GTGCTTGTAA 2040

ATGCCTCCAC GGGCCACCTT CTGTTGCCCA TTGAGAATCC CAGTGGCATG GATCCAGCAG 2100

GTACTGGTAT ACCACCAAAG GCTACCCACA GCCCCTGGTC TTTCCTTTTG TTAACAATCG 2160

TGGCTAGGGA TGCAGACTCG GGGGCCAATG GGGAACTCTT CTACAGCATT CAAAGTGGGA 2220

ATGATGCTCA TCTCTTTTTC CTCAGCCCTT CCTTGGGGCA GCTATTCATT AATGTCACCA 2280

ATGCCAGCAG CCTCATCGGG AGTCAGTGGG ACCTGGGGAT AGTGGTAGAG GACCAGGGCA
2340

GCCCCTCCTT GCAGACCCAA GTTTCATTGA AGGTCGTGTT TGTCACCAGT GTGGACCACC 2400

TAAGGGATTC TGCTCATGAG CCCGGAGTTC TGAGCACACC AGCACTGGCT TTGATCTGCC 2460

TGGCTGTACT GCTGGCCATC TTTGGATTGC TCTTAGCCCT GTTCGTGTCC ATCTGCAGGA 2520

CAGAGAGAAA GGATAATAGG GCCTACAACT GTCGAGAAGC TGAGTCGTCA TACCGCCACC 2580

AGCCCAAGAG GCCCCAGAAA CACATTCAGA AGGCAGATAT CCACCTGGTG CCTGTGCTTA 2640

GGGCCCACGA GAATGAGACT GATGAAGTCA GGCCATCTCA CAAGGATACC AGCAAGGAGA 2700

CACTGATGGA GGCAGGCTGG GACTCTTGCC TGGAGGCCCC CTTCCACCTC ACACCAACCC 2760

TATACAGGAC CCTGCGTAAC CAAGGCAACC AGGGAGAACT GGCAGAGAGC CAGGAGGTAC 2820

TGCAGGACAC CTTCAACTTT CTCTTTAACC ATCCCAGGCA GAGGAATGCC TCCCGGGAGA 2880

ACCTAAACCT TCCTGAGTCC CCACCTGCTG TACGCCAACC ACTCTTAAGG CCTCTGAAGG 2940

TGCCTGGTAG CCCCATAGCG AGGGCGACTG GAGACCAAGA CAAGGAGGAG GCCCCACAGA 3000

GCCCACCAGC GTCCTCTGCA ACCCTAAGAC GACAGCGGAA TTTCAATGGC AAAGTGTCTC 3060

CTAGAGGAGA GTCCGGTCCT CATCAGATTC TGAGGAGCCT GGTTAGGCTC TCTGTGGCTG 3120

CTTTTGCGGA ACGGAACCCG GTGGAGGAGC CTGCTGGGGA CTCTCCTCCT GTCCAGCAAA 3180

TCTCCCAGCT GCTGTCCTTG CTGCACCAGG GCCAATTCCA GCCCAAACCA AACCACCGAG 3240

GAAATAAATA CTTGGCCAAG CCCGGCGGCA GCAGCAGGGG TACCATCCCA GACACAGAGG 3300

GCCTTGTAGG CCTCAAGCCT AGTGGCCAAG CAGAACCTGA CCTGGAAGAA GGGCCCCCGA 3360

GCCCGGAGGA GGACCTTTCT GTAAAGCGAC TTCTAGAAGA AGAGCTGTCG AGCCTGTTGG 3420

ACCCTAATAC AGGTCTAGCC CTGGACAAGC TGAGTCCGCC TGACCCAGCC TGGATGGCGA 3480

GATTGTCATT GCCCCTCACC ACCAATTATC GAGACAACTT GTCTTCCCCC GATGCTACAA 3540

CATCAGAGGA ACCGAGAACC TTCCAGACAT TCGGCAAGAC AGTTGGACCG GGACCCGAGC 3600

TGAGCCCAAC AGGCACGCGC CTGGCCAGCA CTTTCGTCTC GGAGATGAGC TCTCTGCTGG 3660

AAATGTTGTT GGGGCAGCAC ACGGTACCAG TGGAAGCTGC GTCCGCGGCT TTGCGGAGGC 3720

TCTCGGTGTG CGGGAGGACC CTCAGTCTAG ACCTAGCCAC CAGTGGGGCT TCAGCTTCAG 3780

AAGCACAGGG TAGAAAGAAG GCAGCTGAGA GCAGACTTGG CTGTGGCAGG AATCTATGAA 3840

CATGTTTGGT TGGGATGTGT TTGGATCC 3868

(2) INFORMATION FOR SEQ ID NO: 3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 3540 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA to mRNA

(iii) HYPOTHETICAL: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

ATGATGCTAC TTCTGCCATT CCTGCTAGGG CTCTTAGGGC CAGGAAGCTA CTTGTTCATT 60

TCAGGGGATT GTCAGGAGGT GGCCACTGTC ATGGTGAAAT TCCAAGTGAC AGAGGAAGTG 120

CCGTCTGGCA CGGTGATAGG GAAACTGTCC CAAGAACTAA GAGTGGAGGA GAGGCGTGGG 180

AAGGCAGGAG ATGCCTTCCA GATTCTGCAG CTGCCTCAGG CACTGCCGGT TCAGATGAAC 240

TCTGAGGACG GCCTGCTCAG CACTTCCAGC CGGCTGGATC GGGAGAAGCT ATGTCGGCAG 300

GAAGATCCCT GTCTGGTGTC ATTTGACGTG CTTGCCACAG GGCGTCTGC TCTAATTCAT 360

GTGGAGATTC AGGTGCTAGA CATCAATGAC CACCAGCCAC AGTTTCCCAA AGACGAGCAG 420

GAACTGGAAA TCTCAGAGAG TGCCTCTCTG CACACACGAA TCCCCTTGGA CAGAGCTCTT 480

GACCAAGACA CGGGTCCTAA CAGCTTATAT TCCTACTCCC TGTCTCCCAG TGAACACTTT 540

GCCCTGGATG TTATTGTGGG CCCTGATGAG ACCAAACATG CAGAGCTTGT GGTGGTGAAG 600

GAGTTGGACA GGGAACTCCA CTCATATTTT GATCTGGTGC TGACCGCCTA TGACAATGGG 660

AATCCCCCTA AGTCAGGAAT CAGCGTGGTC AAGGTCAATG TCCTGGACTC CAATGACAAT 720

AGTCCAGTGT TTGCTGAGAG TTCACTAGCA CTAGAAATCC CAGAAGACAC TGTTCCTGGT 780

ACTCTTCTCA TAAACCTGAC TGCTACAGAT CCCGACCAAG GACCCAATGG GGAGGTAGAG 840

TTCTTCTTTG GCAAGCATGT GTCCCCAGAG GTGATGAACA CCTTTGGCAT AGATGCCAAG 900

ACAGGCCAGA TCATTCTGCG CCAAGCCCTA GATTACGAGA AGAACCCTGC CTATGAGGTG 960

GATGTCCAGG CAAGGGATTT GGGTCCCAAT TCCATCCCAG GCCATTGCAA AGTTCTTATC 1020

AAAGTTCTGG ATGTCAATGA CAATGCCCCA AGCATCCTCA TCACGTGGGC CTCCCAGACG 1080

TCGCTGGTGT CAGAAGATCT TCCCAGGGAT AGCTTCATTG CCCTTGTCAG TGCGAATGAC 1140

TTGGACTCAG GAAACAACGG TCTCGTCCAC TGTTGGCTGA ATCAAGAGCT GGGCCACTTC 1200

AGACTGAAAA GGACTAACGG CAACACGTAC ATGCTGCTCA CCAATGCCAC ACTGGACAGA 1260

GAGCAGTGGC CCATATATAC TCTCACTGTG TTTGCCCAAG ACCAAGGACC CCAGCCCTTA 1320

TCAGCTGAGA AGGAGCTCCA AATTCAGGTT AGTGATGTCA ATGACAATGC CCCTGTGTTT 1380

GAGAAGAGCC GGTACGAGGT CTCCACTTGG GAAAATAACC CACCCTCTCT TCACCTCATC 1440

ACGCTCAAAG CGCATGATGC TGACTTGGGC AGTAATGGAA AAGTGTCATA CCGTATCAAG 1500

GACTCCCCCG TTTCTCACTT AGTCATTATT GACTTTGAAA CAGGAGAAGT CACTGCTCAG 1560

AGGTCACTGG ACTATGAACA GATGGCAGGC TTTGAGTTCC AGGTGATAGC AGAGGACAGA 1620

GGGCAACCCC AGCTCGCATC CAGCATCTCG GTGTGGGTTA GCCTCTTGGA TGCCAATGAT 1680

AATGCCCCAG AAGTGATTCA GCCTGTGCTC AGTGAAGGCA AAGCCACCCT TTCGGTGCTT 1740

GTAAATGCCT CCACGGGCCA CCTTCTGTTG CCCATTGAGA ATCCCAGTGG CATGGATCCA 1800

GCAGGTACTG GTATACCACC AAAGGCTACC CACAGCCCCT GGTCTTTCCT TTTGTTAACA 1860

ATCGTGGCTA GGGATGCAGA CTCGGGGGCC AATGGGGAAC TCTTCTACAG CATTCAAAGT 1920

GGGAATGATG CTCATCTCTT TTTCCTCAGC CCTTCCTTGG GGCAGCTATT CATTAATGTC 1980

ACCAATGCCA GCAGCCTCAT CGGGAGTCAG TGGGACCTGG GGATAGTGGT AGAGGACCAG 2040

GGCGCCCCTC CTTGCAGACC CAAGTTTCAT TGAAGGTCGT GTTTGTCACC AGTGTGGACC 2100

ACCTAAGGGA TTCTGCTCAT GAGCCCGGAG TTCTGAGCAC ACCAGCACTG GCTTTGATCT 2160

GCCTGGCTGT ACTGCTGGCC ATCTTTGGAT TGCTCTTAGC CCTGTTCGTG TCCATCTGCA 2220

GGACAGAGAG AAAGGATAAT AGGGCCTACA ACTGTCGAGA AGCTGAGTCG TCATACCGCC 2280

ACCAGCCCAA GAGGCCCCAG AAACACATTC AGAAGGCAGA TATCCACCTG GTGCCTGTGC 2340

TTAGGGCCCA CGAGAATGAG ACTGATGAAG TCAGGCCATC TCACAAGGAT ACCAGCAAGG 2400

AGACACTGAT GGAGGCAGGC TGGGACTCTT GCCTGGAGGC CCCCTTCCAC CTCACACCAA 2460

CCTATACAGG ACCCTGCGTA ACCAAGGCAA CCAGGGAGAA CTGGCAGAGA GCCAGGAGGT 2520

ATGCAGGACA CCTTCAACTT TCTCTTTAAC CATCCCAGGC AGAGGAATGC CTCCCGGGAG 2580

AACCTAAACC TTCCTGAGTC CCCACCTGCT GTACGCCAAC CACTCTTAAG GCCTCTGAAG 2640

GTGCCTGGTA GCCCCATAGC GAGGGCGACT GGAGACCAAG ACAAGGAGGA GGCCCCACAG 2700

AGCCCACCAG CGTCCTCTGC AACCCTAAGA CGACAGCGGA ATTTCAATGG CAAAGTGTCT 2760

CCTAGAGGAG AGTCCGGTCC TCATCAGATT CTGAGGAGCC TGGTTAGGCT CTCTGTGGCT 2820

GCTTTTGCGG AACGGAACCC GGTGGAGGAG CCTGCTGGGG ACTCTCCTCC TGTCCAGCAA 2880

ATCTCCCAGC TGCTGTCCTT GCTGCACCAG GGCCAATTCC AGCCCAAACC AAACCACCGA 2940

GGAAATAAAT ACTTGGCCAA GCCCGGCGGC AGCAGCAGGG GTACCATCCC AGACACAGAG 3000

GGCCTTGTAG GCCTCAAGCC TAGTGGCCAA GCAGAACCTG ACCTGGAAGA AGGGCCCCCG 3060

AGCCCGGAGG AGGACCTTTC TGTAAAGCGA CTTCTAGAAG AAGAGCTGTC GAGCCTGTTG 3120

GACCCTAATA CAGGTCTAGC CCTGGACAAG CTGAGTCCGC CTGACCCAGC CTGGATGGCG 3180

AGATTGTCAT TGCCCCTCAC CACCAATTAT CGAGACAACT TGTCTTCCCC CGATGCTACA 3240

ACATCAGAGG AACCGAGAAC CTTCCAGACA TTCGGCAAGA CAGTTGGACC GGGACCCGAG 3300

CTGAGCCCAA CAGGCACGCG CCTGGCCAGC ACTTTCGTCT CGGAGATGAG CTCTCTGCTG 3360

GAAATGTTGT TGGGGCAGCA CACGGTACCA GTGGAAGCTG CGTCCGCGGC TTTGCGGAGG 3420

CTCTCGGTGT GCGGGAGGAC CCTCAGTCTA GACCTAGCCA CCAGTGGGGC TTCAGCTTCA 3480

GAAGCACAGG GTAGAAAGAA GGCAGCTGAG AGCAGACTTG GCTGTGGCAG GAATCTATGA 3540

CLAIMS

What is claimed is:

1. A glycosylated or unglycosylated protein comprising an amino-acid sequence shown in SEQ ID NO:1 or a homologous sequence having at least 70% homology to the sequence shown in SEQ ID NO:1.

2. A cDNA sequence coding for a protein of claim 1.

3. A cDNA sequence of claim 2 having the nucleotide sequence shown in SEQ ID NO: 2.

4. A structural gene coding for a protein of claim 1 or a peptide derived from the protein.

5. A structural gene according to claim 4 having the nucleotide sequence shown in SEQ ID NO: 3.

6. A recombinant protein or peptide expressed by a structural gene or a fragment of the gene of claim 4 or claim 5.

7. An antibody binding specifically to a protein according to claim 1 or a part of the protein.

8. A modifier of the binding of a protein of claim 1.

9. A modifier of claim 8 which inhibits or induces homophilic binding of the protein of claim 1 selected from the group consisting of antibodies which specifically bind to the protein of claim 1, and proteins, peptides,

peptidomimetics and organic molecule-ligands derived from the amino-acid sequence of the protein of claim 1.

10. A diagnostic kit comprising as a diagnostic reagent an antibody of claim 7 or a modifier of claim 8 or claim 9.

11. A vaccine adjuvant comprising a modifier according to claim 8 or claim 9.

12. A medicament comprising as an active ingredient a modifier according to claim 8 or claim 9.

13. A transgenic animal or cell overexpressing or lacking a protein of claim 1.

14. Antisense oligonucleotide based on the cDNA sequence according to claim 2 or claim 3.

15. A monoclonal antibody which specifically binds to a VE-cadherin and modifies angiogenesis.

16. An antibody of claim 15, wherein the VE-cadherin is mammalian.

17. An antibody of claim 15, wherein the VE-cadherin is human.

18. A hybridoma cell line producing a monoclonal antibody of claim 15.

19. A polypeptide which comprises an amino acid sequence which is substantially the same as the amino acid sequence of the variable region of the monoclonal antibody of claim 15.

20. A nucleic acid that encodes the polypeptide of claim 19.

21. A chimeric antibody or a fragment thereof comprising the polypeptide of claim 20.

22. A chimeric antibody of claim 21 comprising an amino acid sequence of a human antibody constant region and an amino acid sequence of a non-human antibody variable region.

23. A chimeric antibody of claim 22, wherein the non-human variable region is murine.

24. A polypeptide which comprises an amino acid sequence which is substantially the same as the amino acid sequence of the hypervariable region of the monoclonal antibody of claim 15.

25. A nucleic acid that encodes the polypeptide of claim 24.

26. A humanized antibody or a fragment thereof comprising the polypeptide of claim 24.

27. The humanized antibody of claim 26 comprising amino acid sequences of framework and constant regions from a human antibody, and an amino acid sequence of a non-human antibody hypervariable region.

28. The humanized antibody of claim 27, wherein the amino acid sequence of the hypervariable region is murine.

29. A method of modifying VE-cadherin activity in cells comprising contacting the cells with the antibody of any of claims 15, 21 and 26.

30. A method of claim 29, wherein the cells are endothelial cells.

31. A method of inhibiting angiogenesis in a mammal comprising administering an effective amount of any one of the antibodies of claims 15, 21 and 26 to the mammal.

32. A method of claim 31, wherein the mammal is a human.

33. A method of inhibiting tumor growth in a mammal comprising administering an effective amount of any one of the antibodies of claims 15, 21 and 26 to the mammal.

34. A method of claim 33, wherein the mammal is a human.

35. A method of inhibiting tumor growth in a mammal comprising administering an effective amount of any one of the antibodies of claims 15, 21 and 26 and a chemotherapeutic agent.

36. A method of claim 35, wherein the chemotherapeutic agent is selected from the group consisting of doxorubicin, cisplatin and taxol.

37. A pharmaceutical composition comprising any one of the antibodies of claims 15, 21 and 26 and a pharmaceutically acceptable carrier.

38. A pharmaceutical composition of claim 37 further comprising a chemotherapeutic agent.

39. Modifiers that specifically bind to the amino acid sequence TIDLRYMSP.

40. A modifier of claim 39 that is a monoclonal antibody.

41. A synthetic peptide comprising the amino acid sequence TIDLRYMSP and is capable of affecting angiogenesis.

42. A synthetic peptide of claim 41 that is capable of inhibiting angiogenesis.

43. A synthetic peptide of claim 41 that is capable of inducing or promoting angiogenesis.

44. A method of inhibiting angiogeneis in a mammal comprising administering to the mammal a effect amount of a modifier of claim 39.

45. A method of inducing or promoting angiogeneis in a mammal comprising administering to the mammal a effect amount of a modifier of claim 39.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US97/20006

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :Please See Extra Sheet.
US CL :Please See Extra Sheet.
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 536/23.5, 24.5; 530/395, 328, 388.2, 387.9, 387.3, 389.1; 424/133.1, 152.1; 514/ 8, 15, 44;

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, DIALOG's Biotech cluster.
cadherin, cDNA, antibody, VE, tumor, angiogenesis, recombinant

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X ---- Y	CAVEDA, L. et al. Inhibition of cultured cell growth by vascular endothelial cadherin (cadherin-5V/VE-cadherin). J. Clin. Invest. August 1996, Vol. 98, No. 4, 886-893, see entire document.	1-9, 15-19, 29-30, 41-42 ---- 20-30
Y	Gussow, D. et al. Humanization of monoclonal antibodies. Meth. in Enzymol. 1991, Vol. 203, pages 99-121, see entire document.	20-30
Y	UCCINI, S. et al. Co-expression of endothelial cell and macrophage antigens in Kaposi's sarcoma cells. J. Pathol. 1994, Vol. 173, pages 23-31, see entire document.	15-30

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
27 JANUARY 1998	23 FEB 1998

Name and mailing address of the ISA/US	Authorized officer
Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231	F. PIERRE VANDERVEGT
Facsimile No. (703) 305-3230	Telephone No. (703) 308-0196

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US97/20006

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	BREIER, G. et al. Molecular cloning and expression of murine vascular endothelial-cadherin in early stage development of cardiovascular system. Blood. 15 January 1996, Vol. 87, No. 2, pages 630-641, see entire document, especially Figure 1.	1-45

A. CLASSIFICATION OF SUBJECT MATTER:
IPC (6):

C07H 21/04; A61K 38/08, 38/16, 39/395; C07K 14/705, 16/28; C12P 21/08; A01N 43/04

A. CLASSIFICATION OF SUBJECT MATTER:
US CL :

536/23.5, 24.5; 530/395, 328, 388.2, 387.9, 387.3, 389.1; 424/133.1, 152.1; 514/ 8, 15, 44